Pollutant Effects on the Microbial Ecosystem

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Genetic diversity of a microbial community will inevitably be affected by environmental stress. However, our understanding of the implications of these effects is limited. Genetic exchange between natural microbial communities appears to be a common phenomenon, mediated by a number of microbial processes (conjugation, transformation, and transduction). These mechanisms of change are presumably adaptations to natural environmental perturbation, e.g., the low levels of antibiotics produced by other organisms. However, anthropogenic influences on the environment may be accelerating genetic change within microbiologic ecosystems, beyond these natural adaptation rates. This article highlights some of the perceived risks to ecosystem health and research questions that need to be addressed. — Environ Health Perspect 102(Suppl 12):45–48 (1994)

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Introduction

Our understanding of the interactions between anthropogenic discharge and natural microbial communities is limited. This is in part due to the difficulties in extrapolating laboratory-based data to a natural ecosystem (*1*). Genetic exchange and selection are readily shown in laboratory cultures. However, the complexity of the natural microbial community, the interactions between species, and the difficulties of *in situ* measurement with minimal disturbance to the system, have made evaluation of microbial response to chemical pollutants extremely difficult. Our advances in molecular techniques have begun to improve our understanding of these interactions (*2*); however, there is still a long way to go.

Some of the important research questions include:

- How do we measure microbial responses to pollutants?
- What is the effect of enviromental stress on microbial diversity?
- How important is the suspected relationship between plasmid-mediated resistance/adaptation to pollutant chemicals and antibiotic resistance?
- Are we creating conditions that promote genetic transfer in the environment, e.g., are we creating conditions that facilitate transfer of so-called virulence factors?
- Are the ocean sediments a vast reservoir of "dormant" pathogens, which may regain viability as the result of anthropogenically induced change?
- Can we use the microbial community as an indicator of environmental stress?

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Responses to Pollutants

Adaptation of a microbial community to environmental stress can involve induction or derepression of enzymes, genetic changes, and/or selective species enrichment (*3*). There is considerable evidence that microorganisms can rapidly adapt to toxic substances in their environment. Bacteria have a number of strategies for reducing the toxicity of their immediate surroundings. These strategies include specific metal efflux systems, binding of metals by extracellular polysaccharides, immobilization intracellularly, or transformation to more volatile or less toxic forms. In many cases these responses appear to be plasmid mediated (*4,5* for reviews). The most extensively studied bacterial resistance system is the enzymatic reduction of Hg^{2+} to Hg^0, which is highly volatile and rapidly diffuses away from the bacterial cell. The details of the expression of the mercuric reductase gene in different organisms is discussed in the above reviews (*4,5*) and is beyond the scope of this article. However, the majority of mercury resistance systems are encoded by plasmids, allowing for conjugative transfer between organisms. Chlorinated hydrocarbons can affect synthesis of nucleic acids and have also been shown to cause mutations in microorganisms (*6*). Biodegradation of these chemicals can occur, primarily mediated by amplification [through selective enrichment, gene transfer and mutation (*3*)] of genes involved in metabolism of these compounds. As with metal resistance, genes for organic pollutant degradation are often contained on plasmids (*7*). A genetic response to addition of pollutants to the environment should result in selection of microorganisms that are capable of adaptation. The inevitable consequence should be a decrease in genetic diversity, as is assumed for higher organisms (*8*). However, at the microbiologic level this is difficult to assess. Tremendous advances in our methodology for measurement of both microbial community structure and function are beginning to change our perception of microbial community dynamics.

Microbial Diversity

Cherry and co-workers (*9*) investigated the effects of both thermal and chemical pollution on freshwater microbial communities. Thermal pollution resulted in increases in bacterial numbers with a decrease in bacterial diversity, consistent with ecological theory. Chemical pollution resulted in decreases in the chromagenic bacterial composition. This is of particular interest because pigmented bacteria appear to be a major component of the microbial community in water distribution systems; and there has been some suggestion that they may be useful as markers for monitoring water quality, due to their association with oligotrophic unpolluted waters (*10*). Duxbury (*11*) reviewed the literature on effect of heavy metals on the ecology of microorganisms. Changes in microbial community structure have been observed, and in some cases reduction in diversity, with the majority of studies focused on algal and fungal populations.

In our own research in New Bedford Harbor, Massachusetts, we have noted an apparent relationship between heavy metal concentrations in marine sediments and rapid reduction in microbial diversity, as

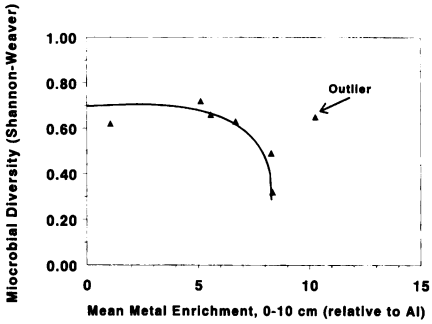

Figure 1. Microbial "plate count" diversity in relation to heavy metal concentrations in New Bedford harbor and Buzzards Bay, Massachusetts. From Ford et al. (*12*).

measured by colony formation on marine agar (Figure 1; *12*). Use of direct plating techniques can only be considered an extremely crude comparison between sites. However, New Bedford Harbor does provide a unique opportunity to study the changing structure and function of microbial communities in response to pollution. There are clear gradients of both organic and metal pollutants from extremely high concentrations in harbor sediments (e.g., 3500 ppm Cu; *12*), out to background levels in Buzzards Bay (<25 ppm Cu).

Antibiotic Resistance

The relationship between genetically determined resistance to pollutants, in particular pesticides and metals, and antibiotic resistance has been suspected for some time (e.g., *13,14*). It is reasonable to assume that this is primarily due to the association of both types of resistance determinants on plasmids. Concern for the connection between metal resistance and antibiotic resistance has recently resurfaced with the connection between dental amalgams and development of antibiotic resistance in human intestinal microbial communities. Work of Poston and Li Saw Hee (*14*) linked resistance to methicillin with mercury, cadmium, and tetracycline resistance in clinical isolates of *Staphylococcus aureus*. However, the environmental information is more limited. Work of Calomiris and others (*13*) suggests that an association of metal tolerance with multiple antibiotic resistance is widespread in drinking water bacteria. Throughout a water distribution system, bacteria are exposed to a variety of metals (e.g., Cu, Pb, and Zn), resulting in developement of tolerant populations. This naturally has serious public health implications since bacteria generally considered harmless may possess resistance determinants that can confer multiple drug resistance.

A related area is the development of disinfection resistance in natural microbial populations. Many of the waterborne disease outbreaks in developed countries (e.g., the outbreak of cryptosporidiosis in Milwaukee, 1993) can be traced to failure of drinking water treatment systems, or leakage within distribution networks. However, an alarming number of outbreaks are caused by development of disinfection resistant strategies. Many of these strategies may be genetically determined, e.g., increased production of extracellular polymers. Polymers increase aggregate and biofilm formation on surfaces, resulting in effectively reducing exposure of the microorganisms to the disinfectant (*15*). Surfaces have been shown by a number of authors to increase rates of transfer of genetic material between microbes (*16*). Surfaces provide protection and nutrient enrichment, and they place microorganisms in close proximity with each other. Within this context, surfaces can be thought of as any interface where nutrients and microorganisms can concentrate. These include solid surfaces, inorganic and organic particulates, the sediment/water and the air/water interfaces. Examples of where anthropogenic influence increases the opportunity for genetic transfer in the environment are discharge of turbid waters, engineered surfaces (bridges, ships, drilling rigs, harbor structures, etc.), and the new interfaces created by oil spills.

Pathogens

The question of enhanced survival and dissemination of pathogens within the environment is exemplified by the recent history of cholera. In the 1990s cholera epidemics (*Vibrio cholerae* O1 serotype) invaded the South American continent, and resurged in Africa and Asia. In addition, multiple drug-resistant strains are now widespread. The most recent cause for alarm is the emergence at the end of 1992 of a *V. cholerae* non-O1 (designated O139 Bengal) that has rapidly spread through India and Bangladesh and is thought to have pandemic potential (*17*). Most cases of this new form of cholera are in adults, suggesting that human populations are immunologically naive and that exposure to *V. cholerae* O1 antigens does not provide immunologic protection. In addition, Islam and co-workers (*18*) report that "Bengal" may be hardier than traditional O1 serotypes. During epidemics of *V. cholerae* O1, these authors report that the bacterium is usually isolated from less than 1% of water samples. With this new epidemic, 12% of water samples yield *V. cholerae* O139. To summarize, the predominant mode of transmission of this pathogen appears to be through water, and it would appear to have a survival advantage over the O1 serotypes. Studies have not yet conclusively shown whether "Bengal" is an *O* antigen mutant of an O1 strain, or a non-O1 strain that has acquired virulence genes by genetic transfer. Natural mutation rates or natural rates of genetic transfer within microbial populations could explain the emergence of this new strain. However, combined with the increases in new cholera strains in Asia, the emergence of other virulent pathogens transmitted through environmental pathways, the widespread misuse of antibiotics in Bangladesh (R Cash, personal communication), and the apparent environmental hardiness of "Bengal," it is urgent that we begin to investigate the links between environmental stress and disease emergence. Certainly one obvious role of global change is becoming clear. Environmental and climatic changes are altering marine ecosystem function. Nutrients and warming are resulting in increases in coastal algal blooms, with resulting increases in plankton productivity. The plankton has been shown to provide a reservoir for *V. cholerae* and other pathogens (*19*).

Methodology

Improvements in nucleic acid technology throughout the 1980s have enabled us to characterize the DNA of natural bacterial and viral populations. Further characterization of the DNA (or RNA) can enable molecular probing for target gene sequences representing specific bacterial metabolic potential (*20*). For example, polymerase chain reaction technology (PCR) is now routinely used to detect presence of the cholera toxin gene in *V. cholerae* samples (*21*). PCR enables detection of specific gene sequences from microorganisms that are present in environmental samples at very low concentrations. Viable but nonculturable stages of a number of pathogens can now be detected with this technology (*22–24*) and indeed, the oceans may represent a reservoir for many of the human pathogens that we have previously thought would rapidly die off in the environment. Work of Paul and others (*2*) suggests that viruses are extremely abundant in marine systems. We are uncertain of their role. However, there is increasing evidence through improved enumeration techniques and electron microscopy for high viral infection rates of

heterotrophic bacteria, estimated to possibly be as high as 70%, contributing from 10 to 100% of bacterial mortality (*25*). If this is the case, viruses may play a crucial role in regulating productivity within the marine ecosystem, completely changing our understanding of trophic dynamics.

Our perception of the importance (and risks) of gene transfer in the environment is dramatically changed by these new measurements of abundance. We create conditions for increased gene transfer through discharge of turbid, often heated, nutrient- and pathogen-enriched waters. The apparent abundance of viruses, and the high bacterial infection rates, suggest that transduction could be a significant route for dissemination of genetic material. Genetic alteration by mutation has been shown to increase under environmental stress (*16*); and, as with infection of higher organisms, viral infection of bacteria may also increase. A key question is whether we can quantify risks from genetic transfer/mutation within a specific (e.g., polluted) aquatic environment.

Evaluations of risk cannot be obtained from laboratory studies alone. There is clear evidence that environmentally induced stress, whether chemical, physical or biologic, dramatically alters the genetic stability of microorganisms. For example, in chromosomal transduction studies, Saye and Miller (*26*) found reversion frequencies of several chromosomal alleles were 50 to 1000 fold higher in a freshwater lake compared to those observed in the laboratory. In addition, in studies on conjugal transfer of plasmids between *Pseudomonas aeruginosa* strains, O'Morchoe and coworkers (*27*) found evidence of considerable increases in genetic instability in a freshwater environment compared to simulated natural conditions.

Direct evidence for an effect of a genotoxic substance (2,4-dichlorophenol) on the structure of natural microbial populations was shown in a study by Short and co-workers (*28*). Addition of a genetically engineered *Pseudomonas putida* to soils amended with 2,4-dichlorophenoxyacetate (2,4-D), resulted in a greater than 400-fold decline in numbers of fungal propagules and a marked reduction in CO_2 evolution. These major changes in the microbial community structure were shown to result from accumulation of 2,4-dichlorophenol, a toxic intermediate in the degradation of 2,4-D. This study serves to highlight the ecologic risks associated with increasing efforts to bioremediate contaminated sites. Addition of engineered microorganisms (or even selective enrichment of indigenous microbes), not only presents risks from genetic transfer (*16*), but can also result in accumulation of metabolites that may be more toxic/genotoxic than the original contaminant.

Summary

To summarize, rapid dissemination of genetic information in microbial communities, whether on plasmids, through viral transduction or even transformation, results in a rapid microbial community response to an anthropogenic influence. The challenge for the microbiologist is to characterize specific genetic markers of the microbial community that reflect anthropogenic influence and can be readily used as indicators of ecosystem health.

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